The Effects of Water on the Rate of Nitrate Nitrogen Fixation by Heterotrophic Bacteria

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Abstract

For our experiment, we studied the effects of water on the nitrogen fixation rates by bacteria. Through a general biota survey, we learned that two sites on the Roland Park campus had statistically significantly different levels of nitrate nitrogen and bacteria. We proposed that in places where the soil contained more water, fixation rates would be higher. We used site three, which was a very dry hillside, and site four, a low wetland, in our experiment. We figured out the difference in the amounts of water contained in the soil from each of the sites, then poured the difference (6/10 of a gallon) onto designated plots in site three. For negative controls, we tested samples of soil in site three and four, both of which were not altered by the change of water supply. After letting the water soak into the soil at site four for a period of time, we collected soil samples from the three different sites and tested them for nitrate nitrogen and performed serial dilutions for bacteria. According to most of our results, our original hypothesis was proven true. Two out of the three important data significance tests showed that our results confirmed that higher water levels in the soil increase the rate at which bacteria fixate nitrogen.

Introduction

Nitrogen is a chemical in soil that is vital to the existence of plants and bacteria and therefore all other living organisms. Bacteria convert nitrite nitrogen to nitrate nitrogen during nitrogen fixation, a process where nitrogen is combined with hydrogen. Plants uses the nitrogen when it incorporates it into macromolecules (proteins and nucleic acids) and vitamins, which enables the plant cells to function properly (Audesirk, 1996). These plants essentially provide the food for all living organisms.

Testing levels of bacteria, nitrite nitrogen, and nitrate nitrogen were all part of the biota survey, completed by the interns of the Environmental Science Summer Research Experience program at the Roland Park School (E.S.S.R.E., 2002). We studied three various plots of land on the school campus that were very different in several ways. Our specific research group studied two sites (site three and four) in particular (see coordinates in Method section). Site three is low wetlands thick with jewelweed whereas site four is on a very dry hill with rhododendrons. As may be expected, these two sites varied immensely as far as their nitrogen and bacteria levels. Once all testing for the biota survey was completed, t-tests were performed to uncover that yes, indeed, there was statistical significance and difference between levels of bacteria and the levels of nitrate nitrogen in these two sites. Our research group decided to investigate these numbers more closely.

The mean nitrate nitrogen level in site four was 25.5 ppm and much less in site three with a mean of 7.04 ppm. We expected that the bacteria growth should follow the same pattern as the nitrate nitrogen since the nitrate nitrogen (as previously stated) can be put into the soil exclusively by bacteria. The bacteria counts, however, did not match the

levels of nitrate nitrogen in the soil. There were more bacteria in site three (with an average of 1.58×10^7 bacteria/ cc soil) than site four (with an average of 4.47×10^6) even though logically, it should have been the reverse. One would presume that where there was high nitrate nitrogen levels there would be high bacteria levels and where there was low nitrate nitrogen levels there would be low bacteria levels. We did not find this to be true.

We then looked at the nitrite nitrogen levels at these sites to see whether they coordinated with the bacteria counts or the nitrate nitrogen levels. Ideally, these three sets of data should be very similar because nitrite nitrogen is the chemical that the bacteria use to convert into nitrate nitrogen. We found that the nitrite nitrogen levels did coordinate with the bacteria levels in both sites. At site three there was 1.58×10^7 bacteria/ cc soil with 0.59 ppm of nitrite nitrogen. Both of these numbers are higher than those from site four with 4.47×10^6 bacteria/ cc and 0.13 ppm of nitrite nitrogen. This told us that in certain locations, the bacteria must have been performing the nitrogen fixation cycle at a slower rate than others.

We wanted to explain this phenomenon for our research project. Possible explanations for these inconsistencies were differences of light, dominant plant species, and most outstandingly, levels of water. We therefore decided that we would study the effect of levels of water on the rate of nitrogen fixation in each site. Because site four was a wetland and at the receiving end of a stream, bacteria may undergo the nitrogen cycle at a faster rate than at site three where there was little water. We tested this by creating very wet areas in site three and comparing the rates that bacteria underwent the nitrogen cycle in these ‘artificial wet’ spots, normal site three soil, and normal site four soil.

Method

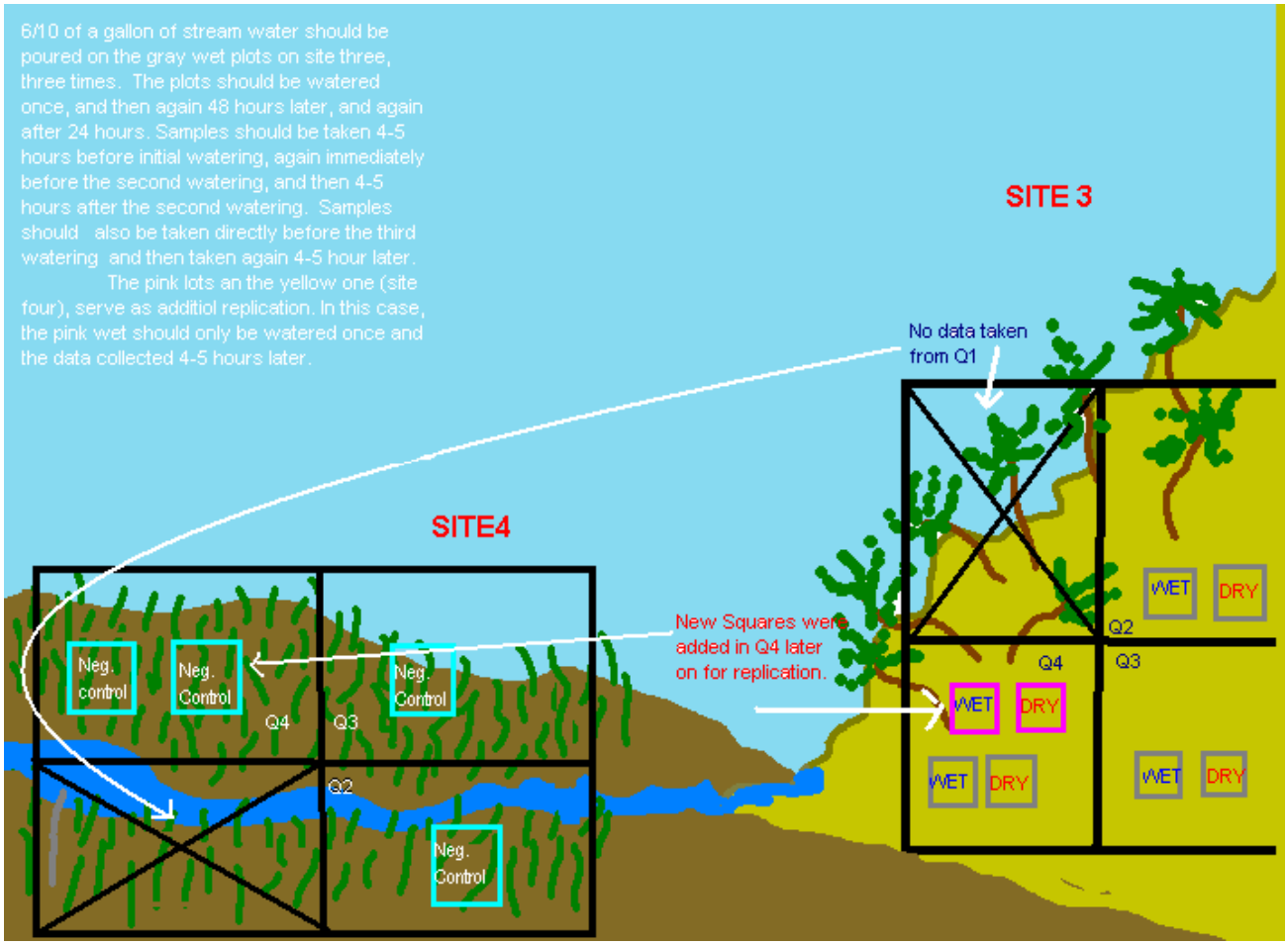
Before we actually performed the experiment, we had to initiate a positive control in order to confirm data we previously observed in a biota survey using the Smithsonian Institutes Biodiversity Protocol (E.S.S.R.E., 2002). We collected three soil samples from quadrants one, two, and three from the two sites we compared. They were site three, a steep heavily forested rhododendron patch, with numerous ferns and dense English ivy located at N 39.35797; W 076.63836, and site four, a wetland monoculture meadow dense with jewelweed located at N 39.35733; W 076.63840. Once we collected the three samples that were about 15 cm long using a soil core sampler 2 cm in diameter from each site, we performed nitrate nitrogen tests on the soil and serial dilutions up to 10^{-3} on the same soil in order to determine the number of bacteria per cm^3 of soil.

In order to determine the difference in the quantities of water within two sites, and also later to determine how much water needed to be added to the soil in site three, one more soil sample was taken from each site. The soil collected was exactly fifteen centimeters long in a two-centimeter wide soil core. The samples were placed in petri dishes and massed then dried in and incubator and massed again in order to determine how much water was in each soil sample. Then once the amount of water for each sample is determined, we found the difference between them. This is the exact formula we used in our calculations: 50*50/ 3.14* 1cm2 = 250/3.14 = 79.58

In order to figure out how much water needed to be added to a half square meter in site 3 to make water levels in both sites equal, we determined the area of the $250cm^2$ in terms of soil core widths. We then multiplied the water difference that we found earlier

with that number and came up with the number of liters needed to be poured on each half square meter, that number was then converted to gallons in order to make it easier to measure and pour the water, we came out with 6/10 of a gallon. Gallon milk jugs were marked to the appropriate amount and then filled to the line using river water from the natural environments in the sites.

Figure 1



In site 3, we then plotted out six square meter plots, 2 in quadrant 2, 2 in quadrant 3, and 2 in quadrant four (see figure 1). In site 4 only one square meter plot per quadrant was needed, since the extra in site 3 were used for control purposes. In one of the plots in each quadrant in site 3 we slowly and evenly poured the appropriate amount of water

from the jug onto the dirt, while breaking up the soil with a trowel so the water was absorbed easier. We did this for two other plots in two other quadrants of site 3. We left the extra plot in each quadrant dry. We left site four untouched.

Four to five hours after the initial watering, we collected a test sample from one of the wet, and dry spots from a plot in site three, and also one from a plot in site four. We used these samples to test for nitrate and set up serial dilutions just to get an idea of where the data was going. After 48 hours we watered the designated plots again. After four or five hours we collected a sample from each square meter plot in the sites, and tested for nitrate and set up serial dilutions. After 24 hours we took samples again, and tested for nitrate and did serial dilutions. We then watered them again, and after about four hours tested and set up dilutions again.

We set up 2 additional half square meters in quadrats 4 of both sites and water one of the plots in site 3. After 4 to 5 hours we collected samples from them and performed the nitrate test on them serial dilutions. This step serves as further replication in the experiment.

We performed nitrate nitrogen test on the soil using the LaMotte© kit method. Before the actual nitrate nitrogen test, we made soil extracts for our soil. The LaMotte© kit method uses universal extraction solution containing a 3% acetic acid, 10% sodium acetate solution. We used the resulting liquid extract to test for nitrate. In the nitrate nitrogen test, 1ml of the extract was mixed with a 25% sodium bisulfate, 7% ammonium sulfate solution. 0.5g of a powder containing 63% barium sulfate, 6% zinc dust, and 1.5% manganous sulfate. We stirred the solution, and left it to stand for 5 minutes. After the

color was fully developed it could be compared to the nitrate nitrogen color chart with predetermined color values to find out the amount of nitrate that is present in the soil. After all tests and bacteria had been counted we compared our data. What we were looking for was an increase in bacteria levels and nitrate levels as the levels of water rise in the wet plots of site 3. Student t-tests and Chi-squared tests were performed to confirm our results.

Results / Discussion

While observing the data (table 1), certain patterns became evident. The Nitrate levels from the dry soil samples are the lowest, while the artificially wet and naturally wet samples have higher levels of nitrate, respectively. Furthermore, while the heterotrophic bacteria counts were harder to follow, in general, the wetter environments had more bacteria.

The important data, however, is really the rate at which nitrate is produced, or fixated by the bacteria. The last columns on the right show these rates. We expected the rates of Nitrate/Bacteria/cm^2 to be higher where there was more water. So, we expected the dry samples to have the slowest rate, while the naturally wet samples to have the most, and the artificially wet samples to be in between these two values but closer to the naturally wet rates. Overall, this proved to be true, and when graphed (see figures 2 & 3) this relationship is easily seen. The graphs show an obvious increase of nitrate per bacteria in wetter environments and an increase in the rate of fixation compared to the wetness of the soil.

Table 1

ESSRE effects of water on nitrogen fixating bacteria

Positive Control:

Date	Site	Sample	Nirate levels (ppm)	Bacteria counts (#/cm^2)	Mean	Rate of N/B
7/18/02	3	a	7.5	1.60E+07		4.69E-07
		b	10	3.00E+07	2.23E+07	3.33E-07
		c	5	2.10E+07		2.38E-07
	4	a	5	5.20E+08		9.62E-09
		b	75	3.00E+07	2.02E+08	2.50E-06
		c	10	5.70E+07		1.74E-07

Artificial Variation and Negitive Control

		Nirate levles (ppm)			Bacteria Counts (#/cm^2)			Nitrate/Bacteria Rate		
		Site 3		Site 4	Site 3		Site 4	Site 3		Site 4
		Dry	Art. Wet	Wet	Dry	Art. Wet	Wet	Dry	Art. Wet	Wet
Date: 7/19/02 Afternoon Data Set A										
Quad.	3	10	5	25	6.30E+07	2.70E+07	3.60E+08	1.59E-07	1.85E-07	1.94E-08
Date: 7/22/02 Afternoon Data Set B										
Quad.	2	0	10	30	9.00E+06	3.60E+07	4.20E+07	0.00E+00	2.78E-07	7.14E-07
	3	0	7.5	10	1.10E+07	1.90E+07	1.00E+08	0.00E+00	3.95E-07	1.00E-07
	4	7.5	25	15	2.00E+07	3.20E+07	3.00E+07	3.75E-07	7.81E-07	5.00E-07

Date: 7/23/02 Morning Data Set C										
Quad.	2	2.5	30	62.5	1.50E+07	4.80E+07	4.00E+07	1.667E-07	6.25E-07	1.56E-06
	3	2.5	5	20	8.00E+06	3.50E+07	3.60E+07	3.125E-07	1.429E-07	5.56E-07
	4	0	7.5	75	1.90E+07	3.20E+07	3.50E+07	0	2.344E-07	2.14E-06
Date: 7/23/02 Afternoon Data Set D										
Quadrat	2	5	10	75	1.70E+07	4.60E+07	5.00E+07	2.941E-07	2.174E-07	1.5E-06
	3	5	10	7.5	8.00E+06	1.40E+07	1.90E+07	6.25E-07	7.143E-07	3.95E-07
	4	2.5	30	75	5.00E+06	2.50E+07	2.10E+07	0.0000005	0.0000012	3.57E-06
	4 new	0	7.5	25	1.40E+07	2.60E+07	5.20E+07	0	2.885E-07	4.81E-07

Figure 2

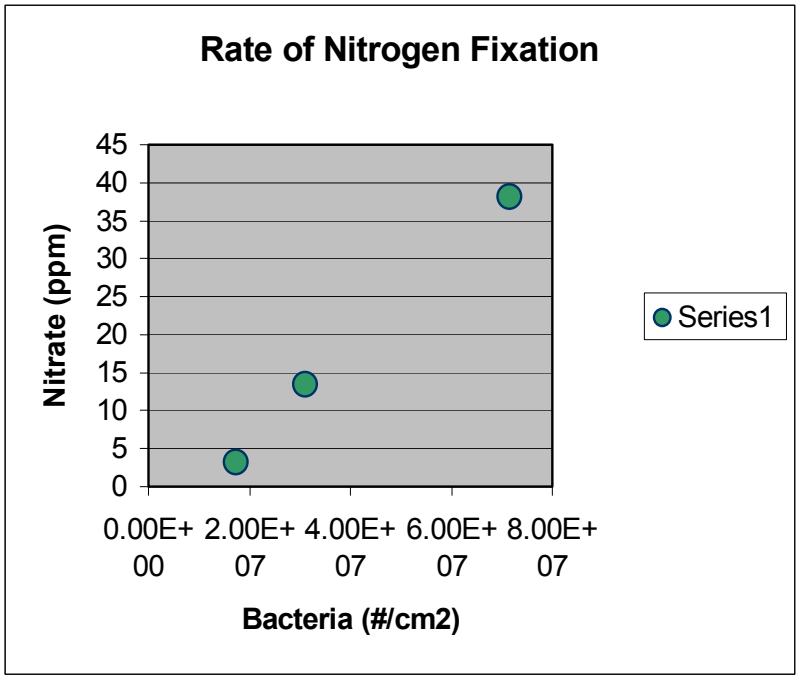
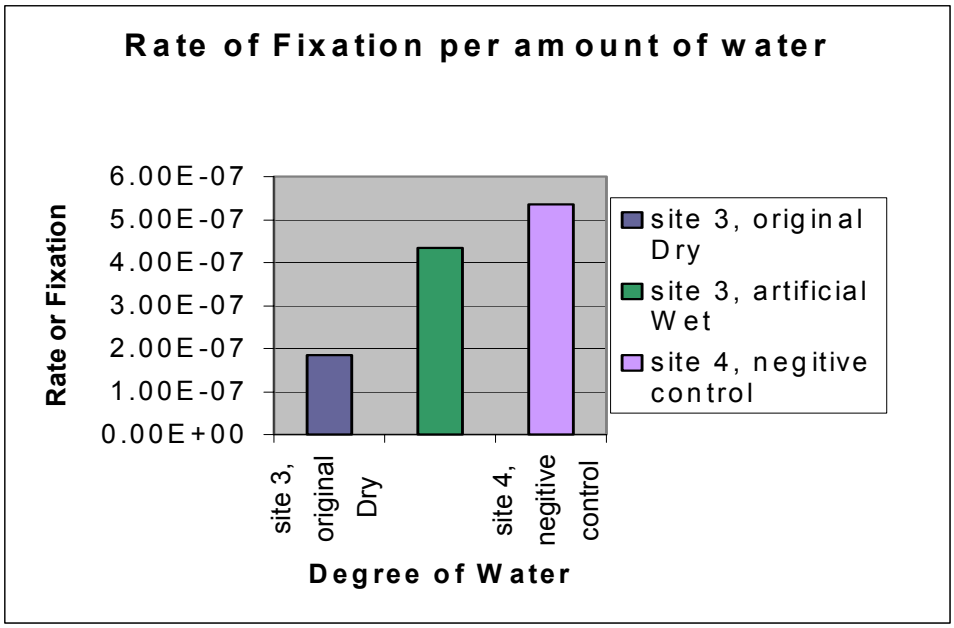


Figure 3



T-tests between the dry and naturally wet samples, the dry and artificially wet samples, and the artificially wet and naturally wet samples were performed. A Chi-squared test was also done, using the artificially wet samples as the observed rates and the naturally wet samples as the expected rates. The results of these tests can be seen below:

	T value	T alpha (.05)	Null accepted?
T-test Dry: Wet	2.52	2.228	No
T-test Dry: art. Wet	1.977	2.228	Yes
T-test Art. Wet: Wet	1.752	2.228	Yes
Chi-test O= Art. Wet, E=Wet	2.1634E-06	18.307	Yes

The purpose of the t-test between the dry and the wet mean fixation rates is to positively confirm that there was a statistically significant difference between those two rates.

The t-test between the dry and the artificially wet rates is to see whether there was a dramatic difference between what the dry rates were and what the watered rates were. According to this t-test, there is not a significant difference.

The result of the t-test between the dry and the artificially wet rates was surprising, so we chose to do a third t-test. This one is between the artificially wet samples and the naturally wet samples. The result was that there was no significant difference between them. This made the data somewhat confusing because it did not follow the pattern of the other results. There was no difference between the artificially wet rates and the naturally wet rates and there was no difference between the dry and naturally wet rates. Theoretically, there should be a difference between the dry and the artificially wet rates even though the t-test showed there was not.

The final test, the chi-squared test, showed that the null hypothesis was accepted. This meant that the artificially wet fixation rates are indeed close to the naturally wet fixation rates that we expected it to be like.

According to the majority of our results, then, our original hypothesis was proven true. Two out of the three important data significance tests showed that our results confirmed that higher water levels in the soil increase the rate at which bacteria fixate nitrogen. It is unusual that the t-test between the dry and the artificially wet sample rates came out the way it did. If there were more samples from replication and repetition, the t-test may have come out differently.

Conclusion

Water levels in the soil affect the rate at which nitrite is converted into nitrate by bacteria through nitrogen fixation. This rate increases when the soil has more water and decreases when the soil is drier.

If we had been allowed more time for our experiment, we would have taken many more samples. We could make new sites and replicate the artificially watered mini-plots, and we could also take more samples from the ones that already exist.

Our research could help science in the future, since we proved our hypothesis and realized something vital about the process of nitrogen fixation. If scientists had to produce nitrate nitrogen from nitrite nitrogen, then our results could help. We found the amounts of water that will allow bacteria to produce the maximum amount of nitrate and so maximum efficiency in another test could be achieved. This could help agricultural research. Since nitrate is vital to the survival of plants, knowing how to produce the maximum nitrate, or be able to produce the maximum rate of nitrogen fixation could help make healthier and stronger plants.

Acknowledgements

We would like to thank Mr. Brock, the coordinator of the ESSRE program for all of the hard work he invested to ensure a wonderful experience for all. We would also like to thank him for putting up with us for the three weeks we worked with him.

Additionally, we would like to thank the National Science Foundation for funding this program that taught us so much and the Roland Park School for allowing us to use their facilities.

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